Express Mail No.: EV335858132US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Kensil

Application No.: 09/760,506

Filed: January 12, 2001

For: INNATE IMMUNITY-STIMULATING COMPOSITIONS OF CPG AND SAPONIN AND METHODS THEREOF

Confirmation No.: 2171

Group Art Unit: 1636

Examiner: Celine X. Qian

Attorney Docket No.: 8449-153-999

DECLARATION OF DR. CHARLOTTE KENSIL UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Charlotte Kensil, Ph.D., do declare and state that:

1. I presently hold the position of consultant at Antigenics, Inc. Antigenics, Inc. is the owner of the entire right, title and interest in, to and under the invention described and claimed in the above-identified patent application by virtue of a chain of title, *i.e.*, from the inventor to Aquila Biopharmaceuticals, Inc. by assignment, and from Aquila Biopharmaceuticals, Inc. to Antigenics, Inc. by merger.

2. I received a Ph.D. from the University of California, San Diego in 1981. My academic and technical experience and honors, and a list of my publications, are set forth in my *curriculum vitae*, attached hereto as Appendix 1.

3. I am the sole inventor of the invention described and claimed in the above-identified U.S. Application No. 09/760, 506 ("the '506 application"). I have read and understand the '506 application, and have been asked to comment on its teaching, in particular, with regard to the enablement of the claimed invention defined by the amended claims, which encompasses a method of treating cancer by using *Quillaja saponaria* saponin including its chemically modified forms in the absence of a vaccine antigen.

4. The following experiments were carried out by me, under my supervision, or in a scientific collaboration with Dr. James Rottman, who was also an employee of Antigenics, Inc. at the time when the experiments were performed. These experiments demonstrated that substantially purified saponin, QS-21, in the absence of a vaccine antigen, is highly effective in treating and preventing cancer in animal models.

5. First, the antitumor effects of QS-21 were evaluated in a Meth A fibrosarcoma mouse model. BALB/c mice (ten mice per group, four groups) were challenged intradermally with 1 x 10^5 live Meth A tumor cells (methylcholanthrene-induced fibrosarcoma cells) on day 0. Phosphate buffered saline ("PBS"), 0.1 μg QS-21, 1 μg QS-21 or 10 μg QS-21 was injected into the Meth A fibrosarcoma allografts 7, 14 and 21 days post-tumor challenge, respectively. Tumor measurements were recorded on days 9, 13, 16, 20, 23, 27, 30, 34 and 37 by measuring the perpendicular tumor diameters.

6. Exhibit A, a graph generated by plotting the average tumor diameter for the ten mice in each group over time, shows the result of the above described experiment. The average tumor diameter in the 10 μg QS-21 group stayed at about 3-5 mm throughout the experiment (even on day 37). However, the PBS group (and the 0.1 μg QS-21 group) showed progressive tumor growth, *e.g.*, about 10 mm on day 27, about 20 mm on day 37 (Exhibit A). This experiment was repeated another time, and a similar result was obtained (see Exhibit B).

7. The Meth A fibrosarcoma studies described in paragraphs 5-6 above demonstrated that QS-21 is highly effective in treating a cancer.

8. The antitumor effects of QS-21 were also evaluated using a P815 tumor model in DBA mice. 1 x 10^5 tumor cells of mastocytoma line P815 were inoculated subcutaneously into the right flank of DBA mice. PBS or 15 μg QS-21 was given to two groups of mice per experiment (ten mice/per group). PBS or 15 μg QS-21 was given in the tumor vicinity (*i.e.*, under the base of the tumor, but not in the tumor itself) two days prior to the tumor transplant, two days after the tumor transplant, and then three times per week afterwards, and tumor growth was monitored for 30 days. The results of the experiment are described in paragraphs 9-10 below.

9. As shown in Exhibit C, at day 20, the mean tumor diameter for the ten mice in the group injected with QS-21 was about 0 mm, while the mean tumor diameter for the ten mice in the group injected with only PBS was about 10 mm. At day 30, the difference in tumor size continued to be apparent: mice injected with QS-21 had a mean tumor diameter of only about 1-2.5 mm, while mice injected with PBS had a mean tumor diameter of about 17-19 mm (Exhibit C).

10. Exhibit D compares the tumor development of each individual mouse (ten mice in each group) between the group that was given PBS ("PBS group") and the group that was given QS-21 ("QS-21 group"). While 9 out of 10 mice in the PBS group developed a measurable tumor by day 30 (Exhibit D(A)), only one out of 10 mice in the QS-21 group developed a measurable tumor by day 30 (Exhibit D(B)).

11. The P815 studies described in paragraphs 8-10 above demonstrated that QS-21 is highly protective against a tumor.

12. In view of the foregoing, I conclude, and others skilled in the art would also conclude, that *Quillaja saponaria* saponin, in the absence of a vaccine antigen, is capable of inhibiting tumor progression *in vivo* when administered to animals with cancer, thereby treating cancer.

13. I hereby declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: December 24, 2003

Dr. Charlotte Kensil

Dr. Charlotte Kensil